latex particles having said $F(ab')_2$ fragments bound thereto, to form a reaction mixture, reaction between said antigen and said $F(ab')_2$ fragments causing agglutination of the latex; and wherein the mixture is observed to determine the extent (if any) of agglutination whereby the presence and/or amount of the said antigen in said fluid sample is determined.

It should be noted that, in latex agglutination tests using $F(ab')_2$ fragments, there should not be any dithiothreitol (DTT) present since this inhibits agglutination. Any DTT can be inactivated by oxidation with hydrogen peroxide.

The method of assay of the invention may, in appropriate cases be effected by continuous flow techniques (which are known in the art) in which individual segments of reaction mixture are passed along the conduit, separated by an inert segment (e.g. air) and, if desired a wash liquid segment. This is described in U.S. Pat. No. 2,797,149 to which reference should be made for further details.

Insolubilised $F(ab')_2$ fragments can be prepared in a number of ways. The fragments may, for example, be absorbed onto a suitable substrate surface. Alternatively, whole immunoglobulin (e.g. IgG) can be covalently bound to a substrate surface and then treated to split off the F(c) fragments, leaving the $F(ab')_2$ fragments covalently linked to the substrate. Thus, $F(ab')_2$ coated latex can be produced in either of these ways, i.e. the latex (having an absorptive coating thereon) can be mixed with $F(ab')_2$ fragments in a buffer, whereupon the $F(ab')_2$ fragments are absorbed directly on to the latex, or whole antibody can be coupled to the latex and then split to release the F(c) fragments (which can then be removed from the mixture), leaving the $F(ab')_2$ fragments bound to the latex. In one example of this procedure, IgG is bound to latex by the method described in our copending U.K. application No. 3238/78 (Case 2090F) to which reference should be made for full details. Briefly, the method comprises first coating the latex with a protein which sticks strongly to the latex and is relatively resistant to proteolysis. An example of such a protein is lactoferrin. The antibodies are then covalently coupled to the lactoferrin using, for example, the Leuchs anhydride of N-ε-chloroacetyl lysine (NCA) as the coupling agent. The latex-lactoferrin-NCA-antibodies are then digested with pepsin, for example under the following conditions: 0.2 M acetate buffer, pH 3.2, pepsin immunoglobulin ratio = 1/10, 0.5% latex suspension, incubation for 60 minutes at 37° C. There is thus obtained latex particles carrying the $F(ab')_2$ fragment of the immunoglobulin.

The latex particles (or other $F(ab')_2$-bearing substrates) obtained in this way, and by the absorbtion method, are not agglutinated by RF levels such as occur in sera rich in RF. The latex particles can be successfully used in the latex agglutination tests for Ag, even in the presence of RF or Clq.

The invention also provides a reagent for use in immunoassay which comprises a suspension of finely divided particulate material having bound thereto the $F(ab')_2$ fragments of an immunoglobulin, the suspension being substantially free from the said immunglobulin and F(c) fragments thereof. In such reagents, the $F(ab')_2$ fragments may for example, be covalently bound or absorbed on the particles. The particles may comprise magnetically attractable material and the fragments may carry an identifying label. The particles may be of any convenient size but generally they will be from about 1 to 30μ. Especially (but not only) in the case of use in continuous flow techniques, the specific gravity of the particles should be from about 1.4 to 3.2 to avoid undue floating or settling of the particles in the reaction mixture.

In order that the invention may be more fully understood, the following Examples are given by way of illustration only.

EXAMPLE 1

Preparation of $F(ab')_2$ latex particles by absorbtion

A 10% suspension of latex particles (Dow, 0.794 micron diameter, S.D. 0.44 micron, No. 41943, Serva Feinbiochemica, D-6900 Heidelberg 1, Germany) is diluted 20 times with 0.02 M glycine/0.035 M NaCl buffer, pH 9.1, and washed once with this buffer. 1/10th Volume of $F(ab')_2$ solution, 2 to 3 mg per ml., prepared as above, is added and after 10 to 15 minutes incubation at room temperature, 1/10th volume of 10% human serum albumin (HSA), in the same buffer as the latex, is added to ensure saturation with protein. After a further 20 to 30 minutes incubation, the latex is washed twice with the original buffer before re-suspending in the original volume of 0.10 M glycine/0.17 M NaCl buffer, pH 9.1, containing 1% HSA, to give a latex suspension of 0.5% (i.e. diluted 20 times compared to the original concentration).

Antiserum

Antiserum to IgE was raised in rabbits using Freund's complete adjuvant and diluted 10 times in clycine buffer containing 3 drops "Tween 20" per liter, and filtered through 0.22 micron Milipore (GWSP 06700) filter before use.

Automated Assay

Patients serum was aspirated at the rate of 0.1 ml. per minute into a continuous flow system comprising a peristaltic pump, manifold, particle counter and recorder. The serum was mixed with 1.0 ml. of glycine buffered latex particles and passed through an incubation coil for 10 minutes. The solution then flowed into a cell counter where the unagglutinated particles were counted, the agglutinated particles being electronically screened out. The concentration of IgE in the serum was directly proportional to the decrease in particles in the range 6 to 100 IU (international units) of IgE. Thirteen patients' samples run repeatedly showed a coefficient of variation of 2% at mid-range and a correlation coefficient of 0.95 when compared with a radioimmunossay test.

EXAMPLE 2

Preparation of $F(ab')_2$ covalently linked to latex

12 mg N-ε-chloroacetyl lysine N-carboxy-anhydride (NCA), dissolved in 100 μl dioxane, was added to 50 mg iron-saturated lactoferrin in 1 ml phosphate buffered saline, pH 7.2 (PBS). After incubation for 24 hours in the dark at 4° C., the preparation was lyophilised for storage. Polystyrene latex particles (0.8μ diameter, 10% suspension) were coated by mixing 500 μg of NCA-lactoferrin with 0.4 ml of PBS and 50 μl of 10% latex. After 45 min incubation at room temperature, the particles were twice washed with 1 ml of 0.2 M carbonate buffer, pH 9.6. To avoid hydrolysis of the chloroacetyl groups at alkaline pH, reduced IgG Ab had to be added immediately. IgG was prepared from rabbit anti-horse